Subacute ruminal acidosis challenge changed in situ degradability of feedstuffs in dairy goats

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ABSTRACT

This study investigated the effects of wheat-induced subacute ruminal acidosis (SARA) on rumen bacterial populations and in situ degradabilities of NDF, starch, and crude protein of feeds. Four multiparous dairy goats (BW = 60 ± 3.3 kg) fitted with ruminal cannulas were assigned to a 2 × 2 crossover design (28-d treatment periods separated by a 7-d washout interval). The treatment diets consisted of 2 levels of cracked wheat: 0 (control, corn based concentrate) and 35% (diet-induced SARA, wheat-based concentrate), with a constant forage- (45% alfalfa hay and 5% corn silage of DM) to-concentrate (50% of DM) ratio. Results indicate that diets with a 35% wheat decreased ruminal pH (6.21 vs. 5.98) and increased the duration (1.13 vs. 4.72 h/d) and area (0.12 vs. 0.78 pH × h/d) of ruminal pH below 5.6 and induced SARA. The SARA increased ruminal total volatile fatty acid concentration, from 105.0 to 123.8 mM, and decreased the acetate molar proportion (62.8 vs. 56.6 mol/100 mol) and the acetate-to-propionate ratio (3.5 vs. 2.8). Compared with the control group, SARA decreases the relative abundance of *Fibrobacter succinogenes* (−59.3%) and *Ruminococcus flavefaciens* (−68.4%), whereas it increased *Succinimonas amylolytica* (198.1%) and *Ruminobacter amylophilus* (125.2%). The SARA decreased 24- and 48-h dry matter (DM) and neutral detergent fiber (NDF) degradabilities of corn silage. The 48-h degradabilities of DM (51.0 vs. 48.2%) and NDF (40.3 vs. 36.0%) in alfalfa hay were not affected by SARA, but the SARA tended to reduce the 24-h DM (49.6 vs. 46.3%) and NDF (37.8 vs. 33.2%) degradabilities. The effective ruminal degradabilities of DM and NDF in alfalfa hay and corn silage were reduced during SARA. In situ degradability parameters of DM and starch of wheat were not affected by SARA, but starch degradability of corn (9.5 vs. 13.3%/h) increased. The SARA reduced in situ 12-h degradabilities of DM and crude protein of soybean meal and extruded soybean with-

out affecting the degradabilities of the other protein supplements (corn gluten meal, cottonseed meal, corn dried distillers grains with solubles, rapeseed meal, and wheat germ meal). These results indicated that the cracked wheat-induced SARA reduced the degradation of NDF in roughages and that of protein in soybean meal (−19.8%) and extruded soy (−18.9%) and increased the starch degradability in corn, due to the increased amylolytic bacteria and decreased cellulolytic bacteria counts in the rumen.

Key words: in situ degradation, protein, starch, subacute ruminal acidosis

INTRODUCTION

Subacute ruminal acidosis is characterized by a long duration of low ruminal pH (pH <5.6 for more than 3 h/d; Gozho et al., 2005), and approximately 20% of high-yielding dairy herds experience SARA (Kleen and Cannizzo, 2012). Subacute ruminal acidosis reduces cellulolytic bacteria counts because of undesirable ruminal pH (<5.8; Russell and Wilson, 1996), resulting in low fiber degradation. Plaizier et al. (2001) observed that SARA reduced in situ 48-h DM (−7%) and NDF (−22%) degradabilities of mixed hay in dairy cows. Several studies reported that ruminants fed highly fermentable carbohydrate diets stimulated the proliferation of ruminal amylolytic bacteria (Goad et al., 1998; Fernando et al., 2010; Petri et al., 2012). With the possible exception of the main cellulolytic species (*Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*), most rumen microorganisms (e.g., *Butyrivibrio fibrisolvens*, *Bacteroides amylophilus*, and *Bacteroides rumenicola*) are proteolytic (Hobson et al., 1982; Wallace, 1996), and ruminants consuming high-grain diets increased proteolytic activity (Furchtenicht and Broderick, 1987; Hristov et al., 2002). In contrast, Rotger et al. (2006) reported that the dietary forage-to-concentrate ratio had no effect on in situ CP degradation kinetics of 7 plant protein supplements in heifers.

The rate and extent of nutrient (starch, protein, and fiber) degradation in the rumen mainly depends on the complex enzymes system (polysaccharide and proteolytic enzymes) of ruminal microbiota and the struc-

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ture of substrates (McAllister et al., 1994; Wang and McAllister 2002; Bach et al., 2005). Factors affecting fiber degradation during SARA have been well studied (Krajcarski-Hunt et al. 2002; Plaizier et al. 2008). However, the interactions between the ruminal amylolytic and cellulolytic bacteria on starch and protein degradation were seldom estimated in the current feed evaluation systems (Bannink and Tamminga, 2005). Data on the effect of SARA on the degradation of starch and protein in the rumen is limited. Therefore, it was hypothesized that SARA might affect the rumen degradation of fiber, starch, and protein in feeds by changing the ruminal bacteria population (increase in amylolytic species and decrease in cellulolytic species). The objective of the current study was to estimate the effect of wheat-induced SARA on ruminal amylolytic and cellulolytic species, and in situ degradability of NDF (in roughages), starch (in grains), and CP (protein supplements) in dairy goats.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

All experimental procedures were approved by the Northwest A&F University Animal Care and Use Committee of P. R. China. Four multiparous Xinong Saanen dairy goats (BW = 60 ± 3.3 kg) fitted with ruminal cannulas were randomly divided into 2 groups (each with 2 goats) according to a 2 × 2 crossover design. Treatment diets consisted of 2 levels of cracked wheat: 0 (1; control) and 35% (2; diet-induced SARA) of dietary DM (Table 1). Goats in group 1 received the control diet and goats in group 2 received the SARA diet in period 1. Goats switched diets in the second period after a 7-d washout period during which all goats were fed the control diet to avoid carryover effects of the SARA diet. Contents of starch and CP in the diet were similar between the treatments. Diets were composed of alfalfa hay (45% of DM), corn silage (5% of DM), and concentrate (50% of DM). Each experimental period lasted for 28 d, with 18 d for adaptation to the diet and 10 d for sample collection, in situ degradation assay, and pH measurements. Goats were housed in individual metabolism cages (1.5 × 0.7 m) and fed twice daily, at 0700 and 1900 h, on an ad libitum basis (allowing for 5–10%orts) with free access to fresh water throughout the experiment.

Sample Collection and pH Measurement

Ruminal pH was continuously monitored for 24 h, from 0700 h on d 19 to 0700 h to d 20. The ruminal pH was measured using an industrial electrode linked to a

pH transmitter and a data logger was used to record data as described in detail by Zhao et al. (2011). Data of ruminal pH were measured at 30-s intervals and averaged every 5 min. The data were summarized across 24 h for each goat as mean, area (time × pH), and time under pH 5.8 or 5.6.

Approximately 100 mL of ruminal fluid was obtained of each goat from the ventral rumen sac at 0700 (before feed delivery), 1000, 1300, 1600, and 1900 h on d 20 and strained through 2-layer cheesecloth. Four milliliters of filtrate were mixed with 1 mL of 25% (wt/vol) metaphosphoric acid and stored at –40°C for analysis of VFA. To extract microbial genomic DNA, samples of rumen content (about 200 g) of each goat were also obtained at each sample time and stored at –80°C.

In Situ Degradation Procedure

Feedstuffs for in situ incubation were dried at 60°C for 48 h and grounded through a 2-mm screen. The chemical composition of the test feeds is presented in Table 2. A portion of each feedstuff (2 g for roughage, 4 g for grain, and 5 g for protein supplement) was weighed into respective nylon bags (7 × 10 cm, 53-μm pore size) and sealed by suture. Bags were soaked in warm water (39°C) for 10 min before being inserted into the rumen. In situ degradation was sequentially determined in roughages (alfalfa hay and corn silage),

Table 1. Ingredients and composition of the diets

Item, % of DM	Control	Subacute ruminal acidosis
Ingredient		
Alfalfa hay	45	45
Corn silage	5	5
Corn	40	7
Wheat	—	35
Wheat bran	—	4.25
Soybean meal	7.5	0.75
Calcium phosphate	0.25	0.25
Limestone	0.75	0.75
Corn oil	—	1
Urea	0.5	—
Salt	0.5	0.5
Vitamin-mineral mix ¹	0.5	0.5
Nutrient composition		
DM	85.7	87.1
NDF	34.3	35.7
ADF	21.7	22.3
Forage NDF	28.5	28.5
CP	15.2	15.3
Starch	28.9	29.2

¹Vitamin-mineral mix (per kilogram, DP104, Wangduofu Biotech Co. Ltd., Yangling, China): 1,000 mg of nicotinic acid, 800 mg of Mn, 1,800 mg of Zn, 2,200 mg of Fe, 370 mg of Cu, 30 mg of Se, 30 mg of I, 50 mg of Co, 6,500 IU of vitamin E, 4,500 IU of vitamin D₃, and 200,000 IU of vitamin A.

Table 2. Chemical composition of in situ incubation feedstuffs

Feedstuff, ¹ % of DM	DM	CP	NDF	Starch	Ash
Corn	96.6	8.3	10.4	63.3	1.6
Wheat	95.8	14.5	13.1	59.1	2.3
Alfalfa hay	92.5	12.2	66.8	—	10.2
Corn silage	93.2	10.8	66.0	—	5.5
Corn gluten meal	96.2	66.9	13.2	—	3.7
Cottonseed meal	95.1	50.5	26.3	—	6.2
Dried distillers grains with solubles	94.4	31.8	32.2	—	6.3
Extruded soybean	97.4	36.9	21.0	—	6.9
Rapeseed meal	95.8	40.1	35.3	—	8.8
Soybean meal	95.1	46.6	14.7	—	7.5
Wheat germ meal	96.1	33.0	28.9	—	2.3

¹These feedstuffs were dried in an oven at 60°C for 48 h before incubating in rumen.

grains (corn and wheat), and plant protein supplements (rapeseed meal, corn gluten meal, cottonseed meal, corn dried distillers grains with solubles, extruded soy, soybean meal, or wheat germ meal) from d 21 (0700 h) to 26 (1900 h). Bags with grain feeds were incubated in the rumen for 0 (water washed), 2, 4, 8, 16, 32, and 48 h. Similarly, bags with roughage were incubated for 0, 3, 6, 12, 24, 48, and 72 h. All bags with protein supplements were incubated for 12 h. All time points were incubated in duplicate. At each time point, 2 bags were removed from each goat and washed under cold tap water until the effluent water remained clear.

Chemical Analyses

Gas Chromatography Analysis. Thawed rumen fluid samples were centrifuged for 15 min at $19,000 \times g$ at 4°C. A sample of 2 mL of supernatant was mixed with 200 μ L of crotonic acid (1% wt/vol; internal standard) and then filtered through a 0.22- μ m filter. Concentrations of ruminal VFA were separated and quantified by gas chromatography (Agilent Technologies 7820 GC system) using a 30-m $\times$ 0.25-mm $\times$ 0.33- μ m fused silica column (AE-FFAP, Atech Technologies Co. Ltd., Lanzhou, China). The injector and detector temperatures were set at 200 and 250°C, respectively. The column temperature was increased from 45 to 150°C at 20°C/min and held for 5 min.

Microbial DNA Extraction and Quantitative Real-Time PCR. Total microbial genomic DNA was extracted from rumen content samples using an E.Z.N.A. Stool DNA Kit (Omega Bio-Tek, Norcross, GA) following the manufacturer's instructions. The method included a bead-beating step for the mechanical lysis of the microbial cells. The quantitative real-time PCR (qPCR) primers of bacteria (*F. succinogenes*, *Rc. flavefaciens*, *Rc. albus*, *Streptococcus bovis*, *Ruminobacter amylophilus*, and *Succinimonas amylolytica*, and general bacteria) used are listed in Table 3. The qPCR assays were performed on a Bio-Rad iQ5 multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA), and the qPCR program and chemicals used were described as Zhao et al. (2013). The relative population abundances of specific bacteria were expressed as the percentage of total bacterial 16S rRNA gene (Denman and McSweeney, 2005).

Starch, NDF, and CP Degradation. After rinsing, all bags were dried in a forced-air oven at 60°C for 72 h to determine DM. For corn and wheat samples, the residual starch content in the bag was determined by an enzymatic method (α -amylase and amyloglucosidase) with a commercial starch analysis kit (Megazyme, Megazyme International Ireland Ltd., Bray, Ireland). For alfalfa hay and corn silage, the residual NDF in the bag was determined according to the method described by Van Soest et al. (1991), with heat stable α -amylase

Table 3. The PCR primers for amplifying target populations

Target species ¹	Forward primer	Reverse primer
General bacteria	CGGCAACGAGCGCAACCC	CCATTGTAGCACGTGTGTAGCC
<i>Fibrobacter succinogenes</i>	GTTCGGAATTACTGGGCGTAAA	CGCCTGCCCTGAACTATC
<i>Ruminococcus flavefaciens</i>	CGAACGGAGATAATTTGAGTTTACTTAGG	CGGTCTCTGTATGTTATGAGGTATTACC
<i>Ruminococcus albus</i>	CCCTAAAAGCAGTCTTAGTTTCG	CCTCCTTGCGGTTAGAACA
<i>Streptococcus bovis</i>	TTCTAGAGATAGGAAGTTTCTTCGG	ATGATGGCAACTAACATAGGGGT
<i>Ruminobacter amylophilus</i>	CTGGGGAGCTGCCTGAAT	CATCTGAATGCGACTGGTTG
<i>Succinimonas amylolytica</i>	CGTTGGGCGGTCATTTGAAAC	CCTGAGCGTCAGTTACTATCCAGA

¹Primers of general bacteria, *Fibrobacter succinogenes*, and *Ruminococcus flavefaciens* referred to in McSweeney and Denman (2007); *Ruminococcus albus* referred to in Wang et al. (1997); *Succinimonas amylolytica* and *Streptococcus bovis* referred to in Stevenson and Weimer (2007); *Succinimonas amylolytica* referred to in Khafipour et al. (2009b).

(A3306, Sigma-Aldrich, St. Louis, MO) and sodium sulfite, and was expressed free of ash. For protein supplements, the residual CP in the bag was determined according to Kjeldahl method (976.05; AOAC, 1990).

Calculation and Statistical Analyses

Variables of in situ degradation kinetics of nutrients (DM and starch for grains; DM and NDF for forages) were estimated using the NLIN procedure of SAS (SAS Institute, 1999) according to the following model (Ørskov and McDonald, 1979):

$$Dt = a + b \times [1 - \exp(-ct)],$$

where Dt = degraded proportion at time t ; a = the fraction that immediately disappears from the bag; b = the fraction that is potentially degradable over time; c = constant rate of degradation of fraction b (h^{-1}); and t = incubation time (h). Effective ruminal degradability (ERD) was determined by

$$\text{ERD} = a + b \times c / (c + kp),$$

where a , b , and c are the degradation constants as described and kp is the passage rate (0.04 h^{-1} for roughages and 0.06 h^{-1} for grains; Offner et al., 2003).

The duplicate in situ degradation data at each sample point of each goat in every period were averaged. The data of bacteria abundance used for the statistical analysis were log10-transformed to normalize their distribution. Data of ruminal pH, DMI, and in situ degradation parameters were analyzed using the MIXED model of SAS (SAS Institute, 1999) according to the following statistical model (Goiri et al., 2010):

$$Y_{ijk} = \mu + S_i + T_j + P_k + \varepsilon_{ijk},$$

where Y_{ijk} = represents the dependent variable; μ = overall mean; S_i = effect of the i th sequence of treatments (i = AB, BA); T_j = effect of the j th treatment (j = control, SARA); P_k = the effect of the k th period (k = 1, 2); ε_{ijk} = the residual error. For analysis of the repeated measurement data (VFA and bacteria), sampling time was added to the previous model with random (goat) and repeated statements (sampling time). In the repeated measures statistical analyses of variables, data were subjected to 3 covariance structures: unstructured, compound symmetric, and autoregressive order 1 covariance. The most desirable covariance structure was determined according to the smallest Schwarz's Bayesian criterion (Littell et al., 1998). Significance was declared at $P \leq 0.05$; trends were discussed at $P \leq 0.10$.

RESULTS

The sequence or the experimental period had no effect on any of the measurements ($P > 0.50$) and is not presented in the tables.

Feed Intake, Ruminal pH, and Bacteria Abundance

The DMI was not affected by treatments (1380 g/d; $P = 0.574$, Table 4). Induction of SARA decreased average daily ruminal pH ($P = 0.047$) and increased duration and area of ruminal pH below 5.8 or 5.6 ($P < 0.05$). The SARA increased concentration of total VFA ($P = 0.010$) and the molar proportions of propionate ($P = 0.004$). The acetate proportion ($P = 0.013$) and the acetate-to-propionate ratio ($P = 0.001$) were decreased during SARA. The molar ratios of butyrate, isobutyrate, valerate, and isovalerate were not influenced by treatment ($P > 0.05$). Compared with the control group, SARA reduced ruminal relative abundance of *F. succinogenes* and *Rc. flavefaciens* ($P < 0.05$), and increased that of *S. amylolytica* and *Rb. amylophilus* ($P < 0.05$). However, the abundance of *Rc. albus* and *S. bovis* were not affected by SARA ($P > 0.05$).

In Situ Degradability

Induction of SARA reduced 24- and 48-h DM and NDF degradabilities of corn silage (Table 5). The 48-h degradabilities of DM ($P = 0.214$) and NDF ($P = 0.094$) in alfalfa hay were not affected by SARA, but the 24-h DM ($P = 0.054$) and NDF ($P = 0.047$; Table 5) degradabilities tended to be reduced. Compared with the control group, SARA also decreased ERD of DM and NDF of alfalfa hay and corn silage ($P < 0.05$).

In situ degradation parameters (a , b , c) and ERD of DM and starch of wheat were not affected by treatment ($P > 0.05$; Table 6). The degradability rates of DM ($P = 0.060$) and starch ($P = 0.040$) of corn increased during SARA, but did not affect the ERD ($P > 0.05$). The SARA decreased 12-h in situ DM and CP disappearance values of soybean meal ($P = 0.011$) and extruded soy ($P = 0.001$), but did not affect the degradation of the other protein supplements ($P > 0.05$; Table 7).

DISCUSSION

Ruminal pH and its daily fluctuation characteristics are important factors in the regulation of microbial activity and the occurrence of SARA (Plaizier et al., 2008; Lechartier and Peyraud, 2011). Diet with 35% cracked wheat instead of corn (DM basis) resulted in an average duration of ruminal pH below 5.6 for more than 180 min/d, which can be considered as SARA in

Table 4. Effect of SARA on DMI, rumen fermentation, and relative abundance of ruminal bacteria

Item	Control	SARA	SEM	P-value
DMI, g/d	1,358	1,402	26.4	0.57
pH				
Mean	6.21	5.98	0.028	0.047
Area under pH 5.8, pH $\times$ h/d	0.44	1.95	0.411	0.001
Area under pH 5.6, pH $\times$ h/d	0.12	0.78	0.127	0.038
pH <5.8, h/d	3.11	9.85	1.443	0.003
pH <5.6, h/d	1.13	4.72	1.136	0.012
Total VFA, mM	105.0	123.8	4.20	0.010
VFA, mol/100 mol				
Acetate (A)	62.8	56.6	1.33	0.013
Propionate (P)	18.0	20.2	0.47	0.004
Butyrate	13.7	14.3	0.21	0.15
Isobutyrate	2.4	3.1	0.21	0.15
Valerate	3.5	2.8	0.41	0.062
Isovalerate	3.1	3.2	0.30	0.69
A:P ratio	3.5	2.8	0.14	0.001
Rumen bacteria, % of 16S rRNA				
<i>Fibrobacter succinogenes</i>	0.939	0.382	0.1091	0.001
<i>Ruminococcus albus</i>	0.489	0.435	0.0854	0.58
<i>Ruminococcus flavefaciens</i>	0.484	0.153	0.1285	0.022
<i>Streptococcus bovis</i>	0.007	0.009	0.0022	0.74
<i>Succinimonas amylolytica</i>	0.157	0.468	0.1117	0.001
<i>Ruminobacter amylophilus</i>	0.330	0.743	0.1803	0.038

ruminants (Gozho et al., 2005; Khafipour et al., 2009a). Previous studies also reported that substitution of dietary wheat or barley for corn decreased the ruminal

pH (Desnoyers et al., 2008; Leddin et al., 2009), which was attributed to the higher degradability of starch in wheat (Offner et al., 2003). The concentration of total

Table 5. Effect of SARA on in situ degradation of DM and NDF in alfalfa hay and corn silage

Item ¹	Control	SARA	SEM	P-value
Alfalfa hay, %				
DM				
a	18.0	18.3	1.57	0.80
b	35.6	32.7	1.23	0.33
c, %/h	9.1	4.7	0.83	0.065
ERD	42.7	35.6	1.35	0.009
24-h degradability	49.6	46.3	1.08	0.054
48-h degradability	51.0	48.2	1.64	0.21
NDF				
a	13.3	12.8	1.10	0.12
b	36.8	32.4	0.75	0.001
c, %/h	7.8	5.0	0.59	0.024
ERD	35.3	31.3	0.96	0.025
24-h degradability	37.8	33.2	2.61	0.047
48-h degradability	40.3	36.0	5.74	0.094
Corn silage, %				
DM				
a	21.1	22.7	3.54	0.63
b	53.6	46.1	3.66	0.041
c, %/h	6.8	4.9	0.47	0.037
ERD	54.8	48.1	1.79	0.007
24-h degradability	59.8	51.6	1.65	0.002
48-h degradability	69.8	59.3	2.18	0.014
NDF				
a	14.0	16.0	2.74	0.45
b	69.4	53.0	3.86	0.039
c, %/h	6.5	4.2	0.75	0.027
ERD	57.0	43.0	1.66	0.006
24-h degradability	57.1	42.4	2.68	0.001
48-h degradability	68.2	55.5	2.91	0.026

¹a = rapidly degradable fraction, b = slowly degradable fraction, c = constant rate of degradation of fraction b; ERD (effective ruminal degradability) = $a + b \times c / (c + kp)$, where kp is the passage rate of roughage (0.04 h^{-1}).

Table 6. Effect of SARA on in situ degradation of DM and starch in wheat and corn

Item ¹	Control	SARA	SEM	P-value
Wheat, %				
DM				
<i>a</i>	66.4	58.9	5.84	0.68
<i>b</i>	27.9	34.0	5.34	0.75
<i>c</i> , %/h	22.0	38.1	6.67	0.28
ERD	88.4	88.3	0.41	0.81
Starch				
<i>a</i>	69.2	64.3	2.23	0.37
<i>b</i>	25.5	29.8	8.05	0.37
<i>c</i> , %/h	43.2	57.5	9.94	0.53
ERD	91.6	91.1	0.33	0.65
Corn, %				
DM				
<i>a</i>	31.6	21.8	3.01	0.045
<i>b</i>	64.1	74.0	3.52	0.052
<i>c</i> , %/h	7.7	10.7	2.16	0.060
ERD	67.7	69.3	1.44	0.87
Starch				
<i>a</i>	26.4	14.4	3.00	0.025
<i>b</i>	76.9	87.5	3.21	0.063
<i>c</i> , %/h	9.5	13.3	0.86	0.040
ERD	73.6	74.7	0.83	0.38

¹*a* = rapidly degradable fraction, *b* = slowly degradable fraction, *c* = constant rate of degradation of fraction *b*; ERD (effective ruminal degradability) = $a + b \times c / (c + kp)$, where *kp* is the passage rate of concentrate (0.06 h⁻¹).

VFA and the molar proportion of propionate increased during SARA, which indicated that more starch was fermented in the rumen compared with the control group.

In the current study, induction of SARA in dairy goats increased some amylolytic (*Rb. amylophilus* and *S. amylolytica*) and reduced cellulolytic species (*F. succinogenes* and *Rc. flavefaciens*) in the rumen. The shift of these bacteria during SARA was the result of the low ruminal pH and high ruminal degradable starch. Several experiments also observed that rumen cellulolytic species dramatically decreased under low ruminal pH when animals received highly fermentable carbohydrate

diets (Khafipour et al., 2009b; Petri et al., 2012; Metzler-Zebeli et al., 2013). Because *Rb. amylophilus* and *S. amylolytica* can use α -linked glucose molecules (i.e., maltose, dextrin, and starch) as fermentable substrates, ruminants receiving high-starch diet can stimulate these species proliferation (Goad et al., 1998; Petri et al., 2012). However, Khafipour et al. (2009b) observed that high dietary grain-induced SARA did not affect or even reduced *Rb. amylophilus* and *S. amylolytica* and increased *S. bovis* (lactate producer) in the rumen. This discrepancy might be related to the severity of SARA, as the low ruminal pH (5.62 h/d below pH <5.6) is also unfavorable for the survival of these bacteria (Hook et

Table 7. In situ 12-h DM and CP disappearance values of plant protein supplements

Item ¹	Control	SARA	SEM	P-value
DM disappearance value, %				
Corn gluten meal	41.6	44.5	2.63	0.19
Cottonseed meal	57.6	55.2	1.05	0.17
Dried distillers grains with solubles	53.2	53.7	2.64	0.84
Extruded soybean	76.2	68.4	2.72	0.002
Rapeseed meal	56.0	60.3	2.29	0.34
Soybean meal	78.3	67.2	3.33	0.001
Wheat germ meal	95.7	95.2	0.32	0.28
CP disappearance value, %				
Corn gluten meal	30.8	34.2	2.59	0.16
Cottonseed meal	62.9	58.6	2.43	0.11
Dried distillers grains with solubles	37.2	39.8	4.71	0.60
Extruded soybean	63.9	51.8	4.16	0.001
Rapeseed meal	58.5	59.6	2.77	0.77
Soybean meal	72.2	57.9	4.02	0.011
Wheat germ meal	96.9	96.6	0.44	0.65

al., 2011). In the present study, ruminal *S. bovis* abundance was low and not affected by SARA. This result is in accordance with previous studies during experimentally grain-induced SARA (Fernando et al., 2010; Petri et al., 2012).

As expected, the SARA decreased in situ roughages degradabilities of DM and NDF, which might be attributed to the decline of ruminal cellulolytic bacteria (*F. succinogenes* and *Rc. flavefaciens*). Similarly, Plaizier et al. (2001) observed that SARA reduced in situ DM and NDF degradabilities of mixed hay (alfalfa and grass hay) in dairy cows. Krajcarski-Hunt et al. (2002) also found that induction of SARA by increasing dietary wheat or barley pellets (25% of DM in TMR) decreased in situ NDF degradabilities of grass hay, legume hay, and corn silage. The SARA did not affect the 48-h DM (49.6%) and NDF (38.2%) disappearance values of alfalfa hay, although this challenge reduced these parameters in corn silage (−15 and −19% for DM and NDF, respectively). This discrepancy might be due to the different potential degradation degradability (sum of rapidly degradable fraction and slowly degradable fraction) of the 2 roughages. Alfalfa hay had lower potential degradation fractions of DM (52.3 vs. 71.8%) and NDF (47.7 vs. 76.2%) than corn silage in our study. Because of the low amount of available DM and NDF in alfalfa hay, the time to reach degradation kinetics plateau was shortened, and resulted in similar degradabilities at 48 h. These results suggested that SARA was successfully induced by increasing dietary cracked wheat to 35% in dairy goats, which led to a reduction in fiber degradability and ruminal cellulolytic bacteria (*F. succinogenes* and *Rc. flavefaciens*).

The ERD of starch in wheat and corn was not affected by treatment, although SARA increased the abundance of *S. amylolytica* and *Rb. amylophilus*. The rate and extent of starch degradation in the rumen mainly depends on rumen microorganisms, grain type, processing method, and rumen passage rate (Svihus et al., 2005; Huntington et al., 2006). Data on the effects of ruminal pH or SARA on starch in situ degradation is limited. Several experiments observed that an increased dietary grain content increased ruminal amylase activity in vivo and in vitro (Jouany and Martin, 1997; Martínez et al., 2010). Induction of SARA in our study accelerated the starch degradability rate of corn (9.5 vs. 13.3%/h), which might be attributed to the high abundance of ruminal amylolytic bacteria. However, the in situ degradation rate of starch in wheat was not affected by diets (50.4%/h). Rumen bacteria more easily digest starch in wheat than that in corn because of the different endosperm types (floury endosperm vs. vitreous endosperm; Allen, 2012). Ljøkjel et al. (2003) found that neither pelleting nor expanding had

significant effects on the in situ degradation of starch in wheat and barley, which can be explained by the very high degradability before processing (Svihus et al., 2005). Thus, lack of significant effects of SARA on degradability of starch in wheat might due to its high ruminal degradability.

For plant protein supplements, the 12-h incubation was adopted because this length represents the residue that escapes ruminal degradation (Gargallo et al., 2006). In the present study, SARA reduced DM and CP 12-h in situ disappearance values of soybean meal (−19.8%) and extruded soybean (−18.9%). Previous studies indicated that plant protein supplements have higher degradability in high-roughage diets than in high-concentrate diets (Loerch et al. 1983; Molero et al. 2004). Molero et al. (2004) observed that heifers receiving high-concentrate diets reduced ERD of soybean meal (−6.5%), corn gluten feed (−10.2%), and fish meal (−6.9%) compared with those receiving low-concentrate diets. Kohn and Allen (1995) also found that CP degradability increased with the addition of cellulases in vitro. Plant proteins are integrated within the cellulose tridimensional structure, which must be broken down by polysaccharide enzymes (e.g., cellulase and hemicellulase) of ruminal bacteria to allow proteolysis (Wallace et al., 1997; Bach et al., 2005; Rotger et al. 2006). In the current study, it is possible that SARA decreased ruminal cellulolytic activity and led to a reduction in the degradation rate of protein (soybean meal and extruded soy) due to the limitation of the access of proteases to their target.

In the present study, however, the CP disappearance of the other protein supplements was not decreased during SARA. Loerch et al. (1983) also observed that the in situ degradability of CP in corn gluten meal was not affected by increasing dietary high-moisture corn from 20 to 80% (ruminal pH from 6.55 to 5.75), although the CP degradation of soybean meal (−31.2%) and dehydrated alfalfa (−50.7%) were dramatically reduced. The degradation rate of protein supplements in rumen mainly depends on their structure (fiber-bound protein, susceptibility, and accessibility of peptide bonds; Bach et al., 2005) and the proteolytic activity of ruminal microflora. Therefore, the different CP disappearance among plant protein supplements in response to SARA may be attributed to their different protein to fiber structure.

CONCLUSIONS

The results of the present study indicate that the cracked wheat-induced SARA affected starch (corn) and CP (soybean meal and extruded soy) degradation in the rumen. This effect depended on the structure

of the feed and the ruminal microbiota (i.e., increases in *Rb. amylophilus* and *S. amylolytica* and decreases in *F. succinogenes* and *Rc. flavefaciens*). Future feed evaluation systems need to consider the changes in the degradability of starch and protein in diets when the animal is at risk of SARA.

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